Indicaciones de técnicas complementarias en el diagnóstico y pronóstico de la patología cervical: **lesiones escamosas**

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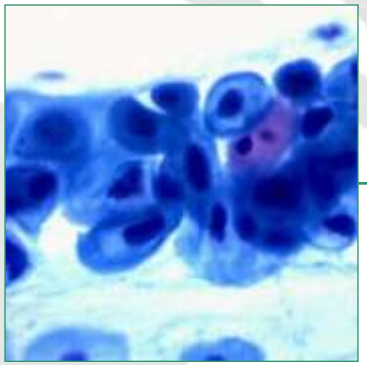
ucb

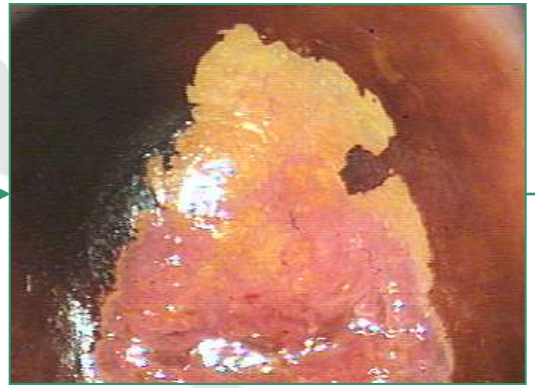
Prevención del cáncer cervical

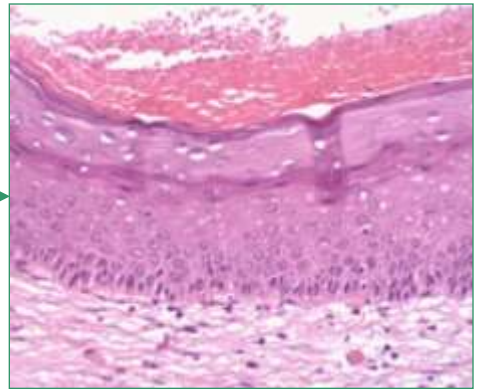
Cribado

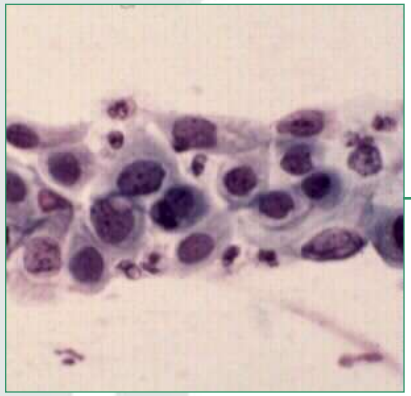
Extensión de la lesión
Selección del sitio de bx

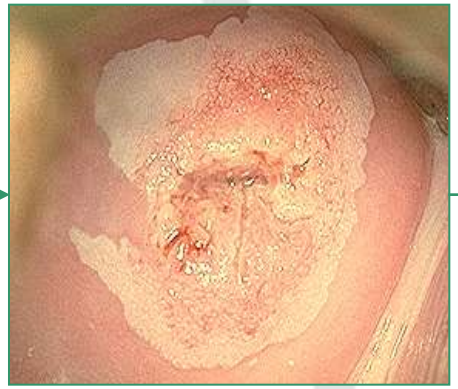
Diagnóstico

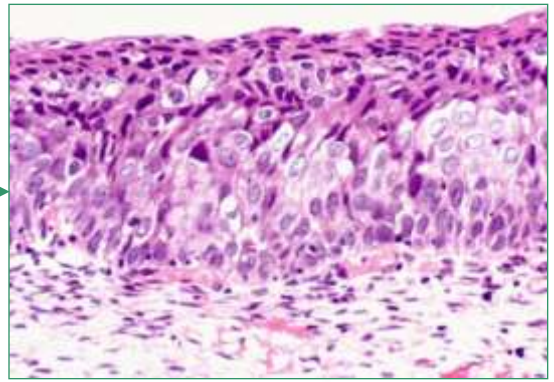












Biopsia cervical: limitaciones

- Importante variación inter e intra- observador en el diagnóstico de CIN2-3

- No permite identificar aquellas lesiones de bajo grado con riesgo de progresión

Concordancia diagnóstica

Author	Year	Interobserver kappa (weighted)	Intraobserver kappa (weighted)
Cocker et al.	1968	0.26-0.30	0.82
Ringsted et al.	1978	0.81	0.82
Bellina et al.	1982	0.24-0.71	0.66
Ismail et al.	1989	0.78	
De Vet et al.	1995	0.71	
McCluggage et al.	1996		0.20-0.54

0.21-0.40	**Fair**
0.41-0.60	**Moderate**
0.61-0.80	Substantial
0.81-1.0	Almost perfect

Revised in Malpica A, et al.
Gynecol Oncol, 2005; 99: S38

Concordancia diagnóstica

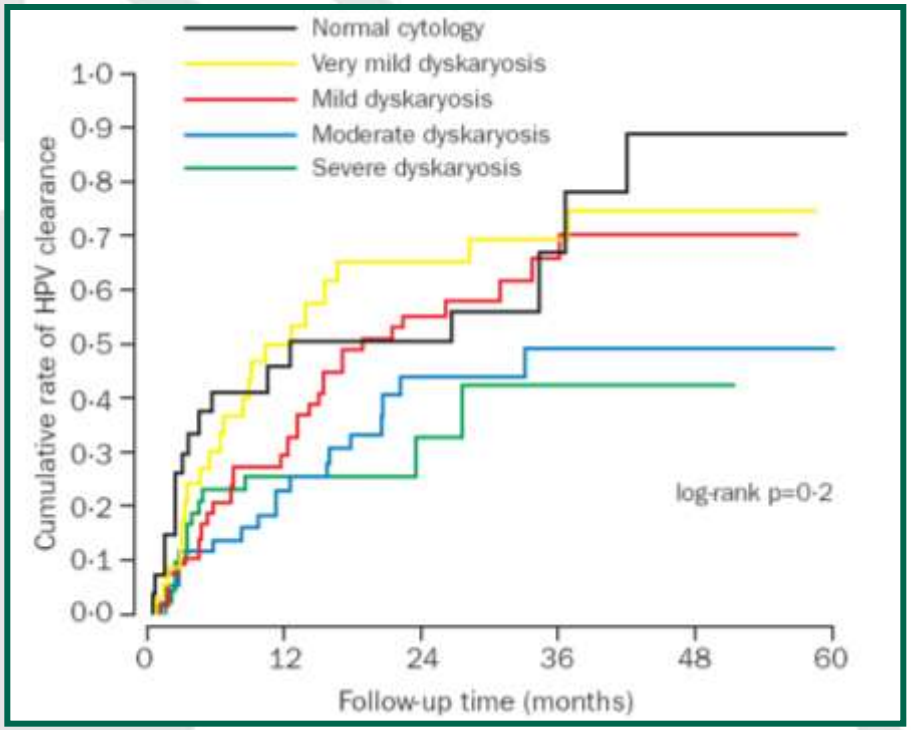
	Normal	NDL	CIN 1	CIN 2	CIN 3	CxCa.	Totals	Concordance
Normal	**738 (71%)**	108 (10%)	115 (11%)	28 (3%)	37 (4%)	10 (1%)	1036	738 (71%)
NDL	108 (68%)	**4 (3%)**	19 (12%)	6 (4%)	13 (8%)	6 (4%)	156	4 (3%)
CIN 1	115 (27%)	19 (4%)	**222 (52%)**	34 (8%)	30 (7%)	4 (1%)	424	222 (52%)
CIN 2	28 (8%)	6 (2%)	34 (10%)	**122 (35%)**	147 (42%)	11 (3%)	348	122 (35%)
CIN 3	37 (4%)	13 (1%)	30 (3%)	147 (16%)	**652 (72%)**	25 (4%)	904	652 (72%)
CxCa.	10 (1%)	6 (1%)	4 (1%)	11 (2%)	25 (3%)	**876 (94%)**	932	876 (94%)

Klaes et al, *Am J Surg Pathol* 2002 26:1389

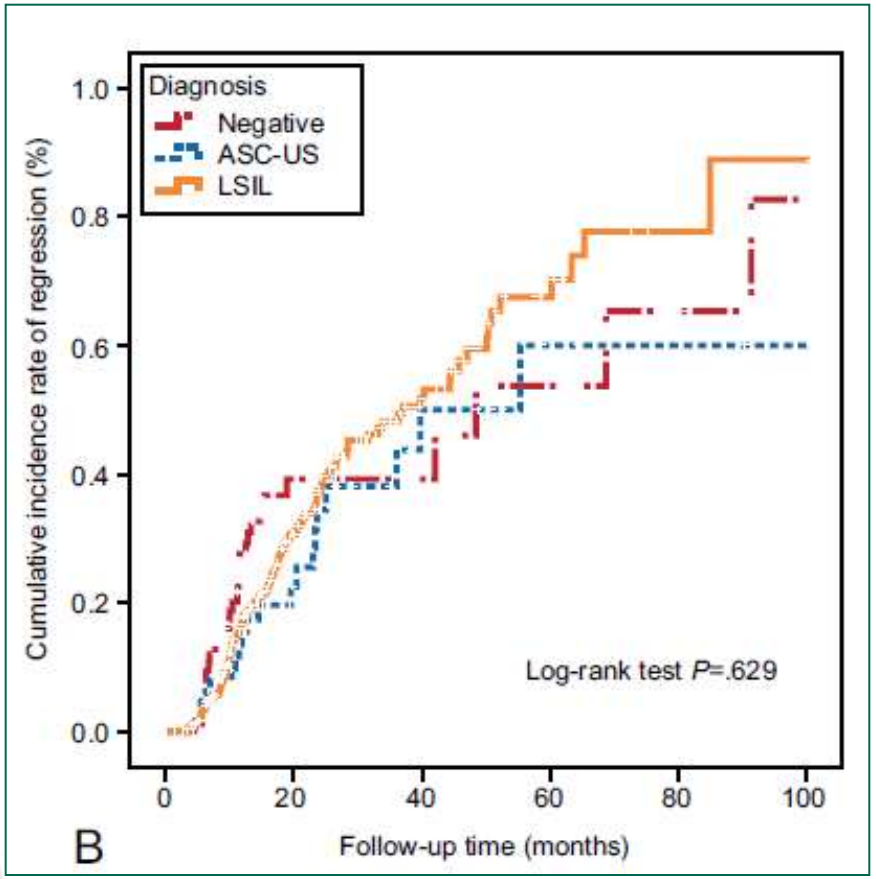
	Community Diagnosis					
	Neg	CIN1	CIN2	CIN3/ AIS	Cancer	Total
Consensus biopsy diagnosis						
Neg						
n	653	88	6	1	0	748
% col	86.5%	19.5%	4.1%	1.1%	0.0%	
CIN1						
n	90	279	23	2	0	394
% col	11.9%	61.9%	15.6%	2.2%	0.0%	
CIN2						
n	10	77	70	20	0	177
% col	1.3%	17.1%	47.6%	21.7%	0.0%	
CIN3/AIS						
n	2	7	48	69	1	127
% col	0.3%	1.6%	32.7%	75.0%	16.7%	
Cancer						
n	0	0	0	0	5	
% col	0.0%	0.0%	0.0%	0.0%	83.3%	
Total	755	451	147	92	6	1451

Galgano MT, et al, *Am J Surg Pathol* 2010 34:1077

Progression and regression



Nobbenhuis et al. *Lancet* 2001; 358: 1782–83



Del Pino et al. *Obstet Gynecol*, 2010; 116: 1324-1331

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¿Cómo reducir la variabilidad inter e intraobservador?

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Condiciones buen biomarcador

- Elevada sensibilidad para CIN2+ y/o CIN3+
- Alta especificidad
- Evaluación fácil y reproducible

Marcadores IHQ-ISH

- p16^{INK4a}
- Ki67 (MIB1)
- HPV L1
- nm 23
- Hibridación *in situ* para VPH
- TOP2A
- MCM2
- ProEx C (TOP2A+MCM2)

$p16^{INK4a}$ in uterine cervix

	Positive p16 staining	
Study	**CIN2+**	**Negative biopsies**
Klaes (*Am J Surg Pathol* 2002)	100%	12%
Hariri (*Int J Gynecol Pathol* 2007)	100%	6%
Benevolo (*Histopathology* 2010)	96%	19%
Ordi (*Int J Gynecol Pathol* 2009)	99%	0%
Galgano (*Am J Surg Pathol* 2010)	87%	5%

p16^{INK4a} in CIN3+

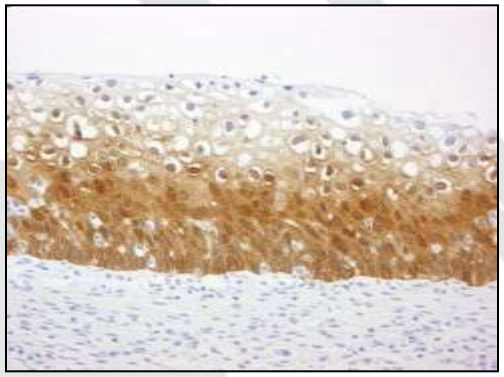
Study	Sensitivity	Specificity
Klaes (*Am J Surg Pathol* 2002)	100%	62%
Wang (*Cancer Epidemiol Prev* 2004)	100%	95%
Galgano (*Am J Surg Pathol* 2010)	99%	74%
Guo (*Am J Clin Pathol* 2011)	90%	71%

p16^{INK4a} in CIN2+

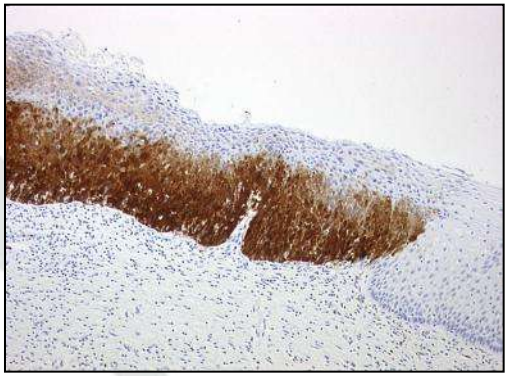
Study	Sensitivity	Specificity
Klaes (*Am J Surg Pathol* 2002)	100%	71%
Wang (*Cancer Epidemiol Prev* 2004)	81%	95%
Hariri (*Int J Gynecol Pathol* 2007)	100%	72%
Kong (*Am J Surg Pathol* 2007)	82%	100%
Ordi (*Int J Gynecol Pathol* 2009)	99%	89%
Benevolo (*Histopathology* 2010)	96%	66%
Galgano (*Am J Surg Pathol* 2010)	87%	83%
Guo (*Am J Clin Pathol* 2011)	79%	85%

p16^{INK4a} Immunostaining

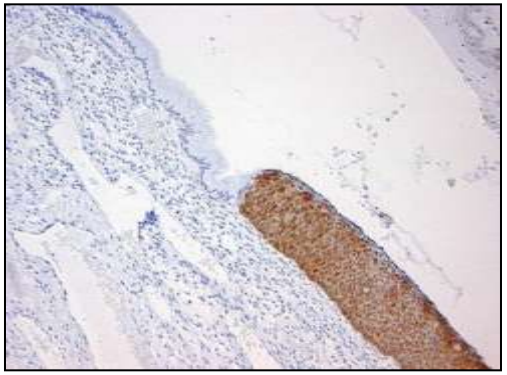
- Diffuse Staining patterns (Positive result)



CIN 1

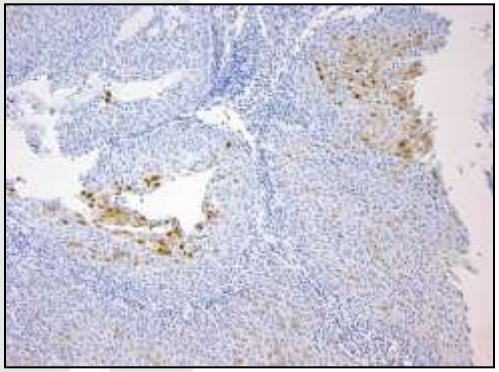


CIN 2

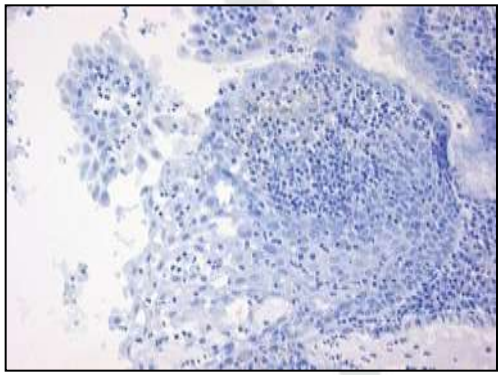


CIN 3

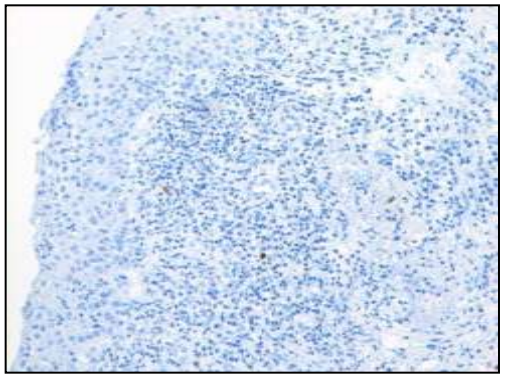
- Focal/Negative Staining patterns (Negative result)



Mature metaplasia



Immature metaplasia



Cervicitis

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CINtec® Study; Design (I)

- Gold standard diagnosis
  - 3 Expert gynecopathologists, independently reading all 500 H&E slides
  - Any discrepant result subjected to adjudication meeting
  - **Consensus diagnosis on H&E slides** only during adjudication meeting as the final **Gold Standard Diagnoses**

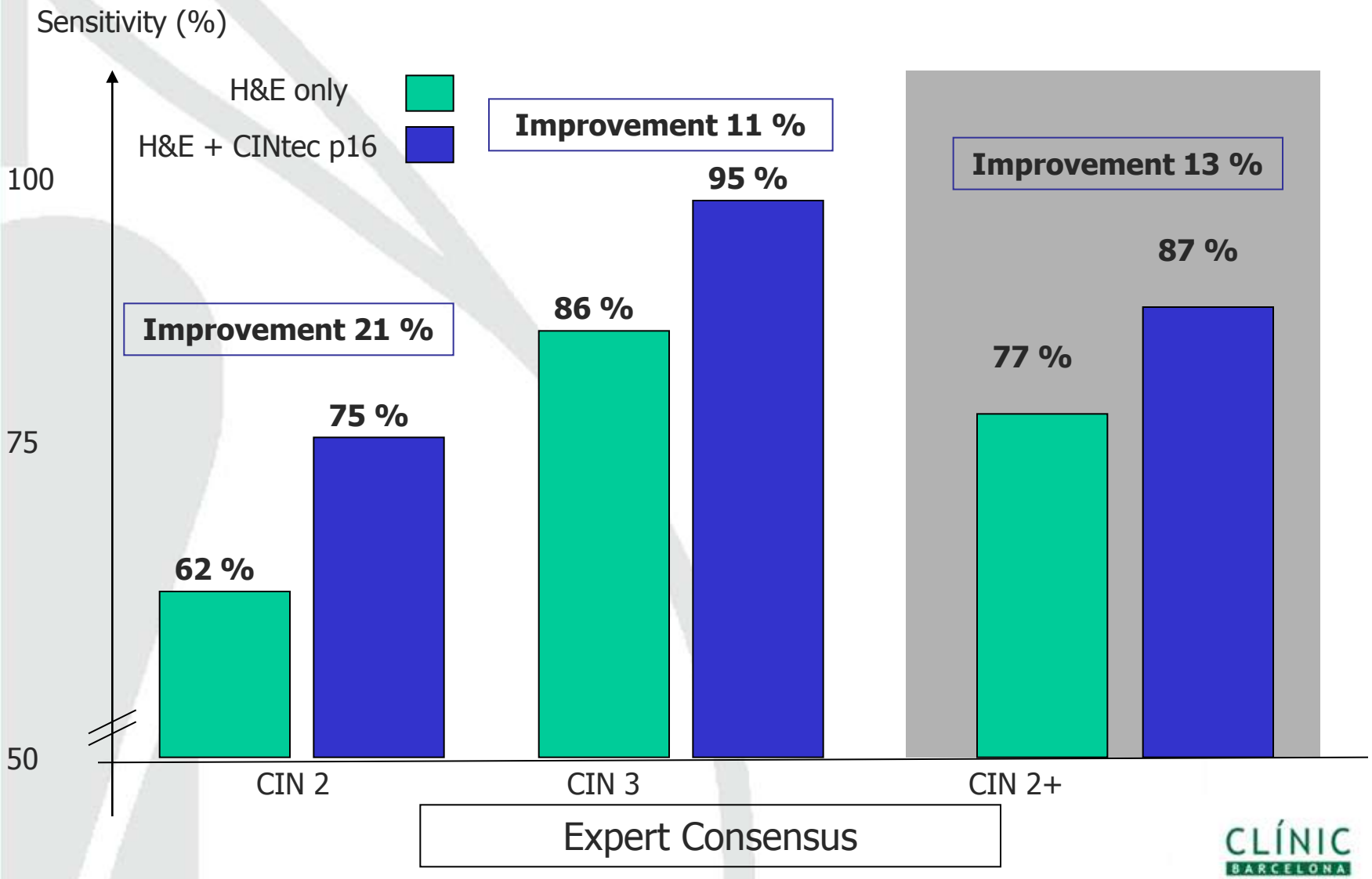
Bergeron C, Ordi J, et al. *Am J Clin Pathol* 2010; 133:395

CINtec® Study; Design (II)

- Study Panel Diagnoses (12 general pathologists)
  - Diagnosis of all 500 HE slides
  - Washout periode of ≥ 4 weeks
  - New diagnosis on the same set of 500 HE slides, conjunctively with the matched p16 stained slides
  - Panel pathologists blinded to case numbers, original diagnoses, and Gold standard diagnoses

Bergeron C, Ordi J, et al. *Am J Clin Pathol* 2010; 133:395

p16^{INK4a}: Gain in Sensitivity



Inter-Observer Agreement

Diagnostic Category	Kappa H&E	Kappa H&E plus CINtec® Histology	Statistical significance
CIN2+, all cases	0,580	0,756	p<0,0001
CIN2+, punch biopsies only	0,598	0,748	p<0,0001
CIN2+, conization biopsies only	0,548	0,765	p<0,0001

Bergeron C, Ordi J, et al. *Am J Clin Pathol* 2010; 133:395

Reproducibility of Rating CINtec®

- Kappa analysis for reproducibility of rating the CINtec® Histology immunostaining pattern as either negative or positive
  - Mean Kappa value of 0,899 (Median 0,903)

Bergeron C, Ordi J, et al. *Am J Clin Pathol* 2010; 133:395

CINtec® Study; Conclusions

- The conjunctive use of $p16^{INK4a}$ in cervical tissue slides leads to a
  - Statistically significant Increase in diagnostic accuracy for diagnosing CIN2+
    - Overall increase of 13% in sensitivity for CIN2+
    - Overall 45% reduction of false negative cases
  - Statistically significant Increase in inter-observer agreement for diagnosing CIN2+

Bergeron C, Ordi J, et al. *Am J Clin Pathol* 2010; 133:395

HPV-p16 en lesiones ocultas

- Identificación de pacientes con estudio simultáneo
  - Biopsia
  - Detección de VPH (HC2)

- 1253 mujeres (1999-2005)

Ordi J, et al. *Int J Gynecol Pathol* 2009; 28:90-97

HPV-p16 en lesiones ocultas

- **Grupo problema:** 139 mujeres
  - Biopsia negativa y VPH simultáneo +
- **Grupos control (50 en cada grupo)**
  - Grupo D: Biopsia negativa y VPH – (496)
  - Grupo E: CIN 1 y VPH+ (248)
  - Grupo F: CIN2-3 y VPH+ (277)

Ordi J, et al. *Int J Gynecol Pathol* 2009; 28:90-97

Biopsia cervical - p16^{INK4a}

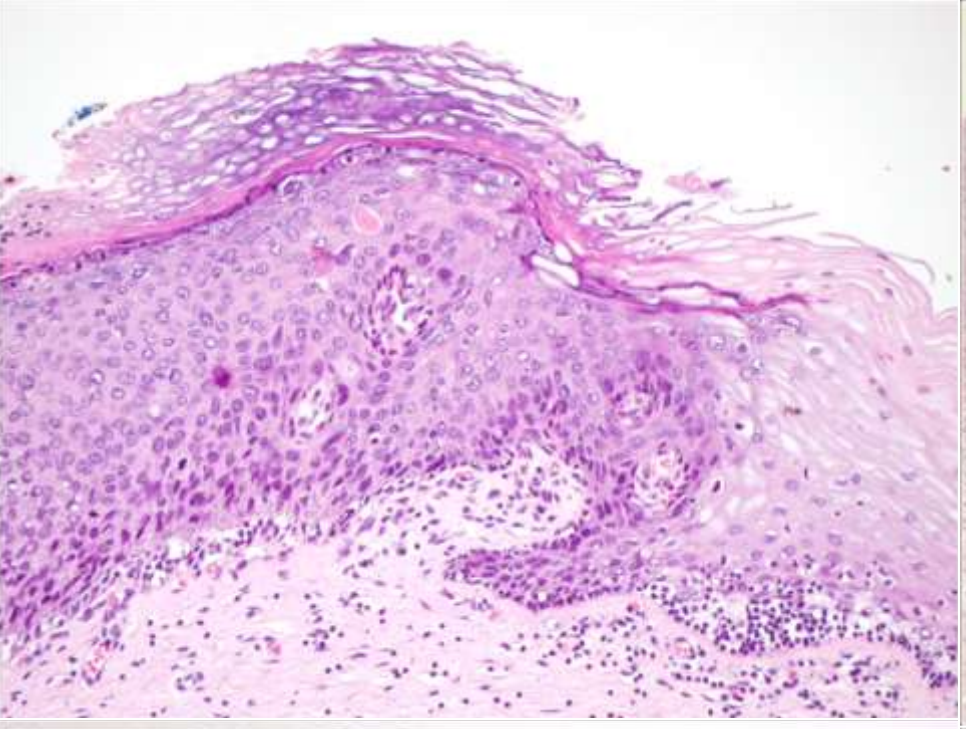
		Biopsy final evaluation			
P16^{INK4a}	**n**	**No lesion (n=107)**	**CIN 1 (n=13)**	**CIN 2/3 (n=19)**	**P value**
Negative	105	103 (98%)	2 (2%)	0 (0%)	<0.001
Focal	10	4 (40%)	6 (60%)	0 (0%)	<0.001
Diffuse	24	0 (0%)	5 (21%)	19 (79 %)	<0.001

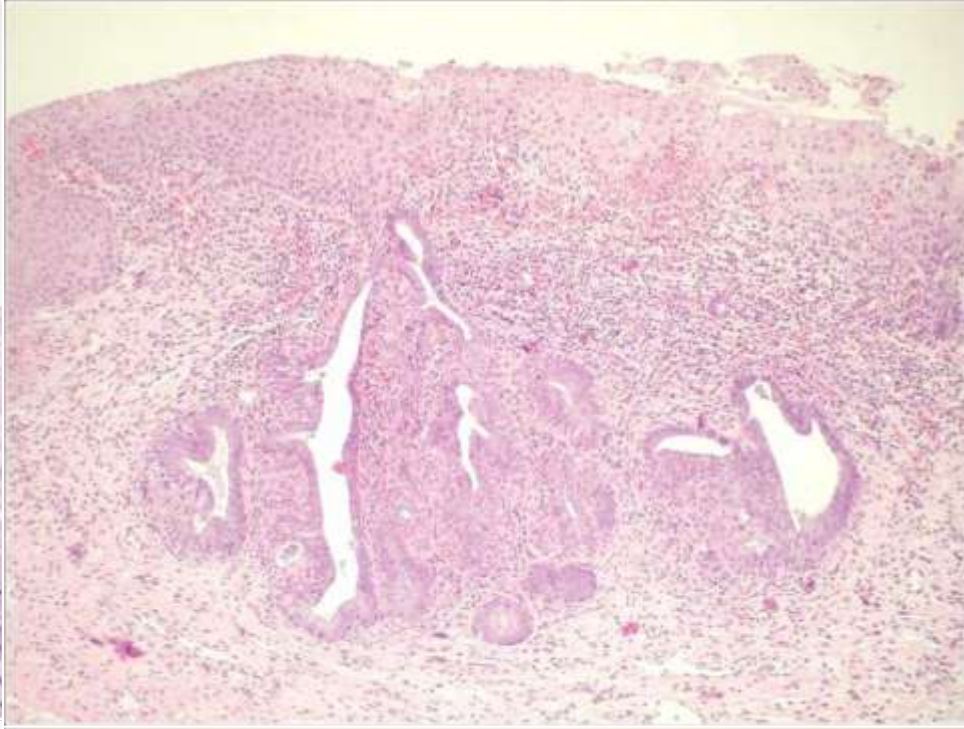
Ordi J, et al. *Int J Gynecol Pathol* 2009; 28:90-97

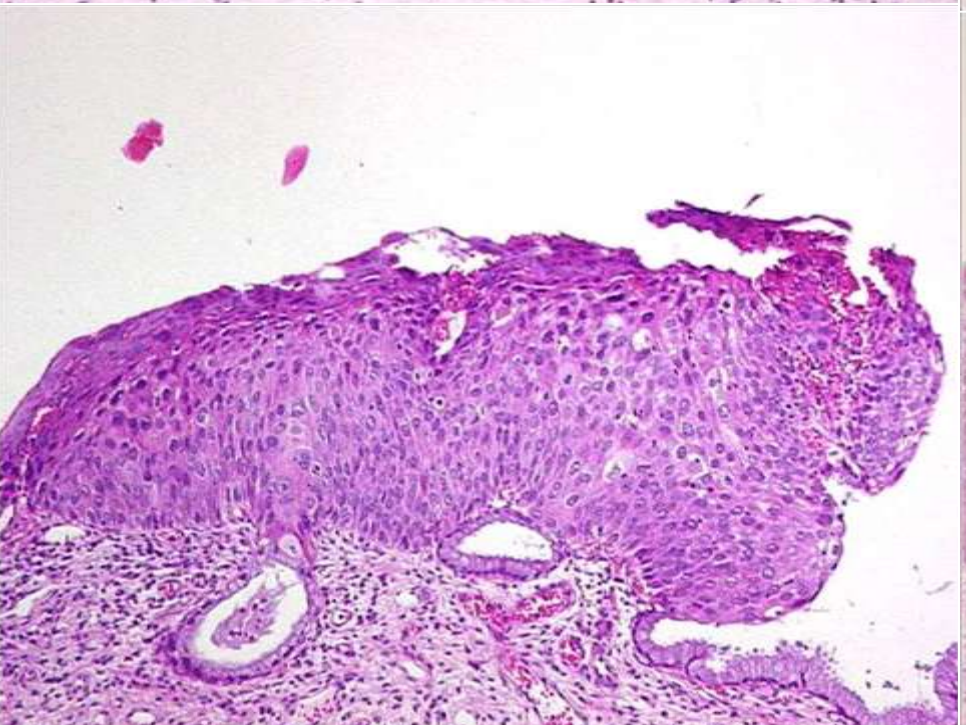
Lesión cervical - p16^{INK4a}

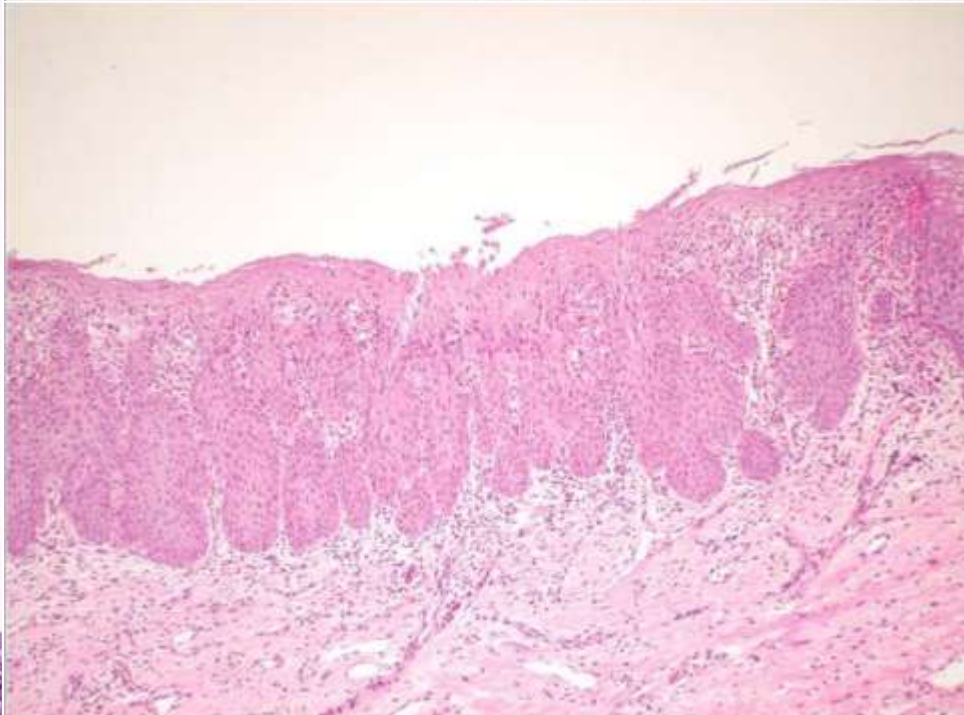
		P16^{INK4a} (%)		
Biopsy final evaluation	**N (326)**	**Negative (n=182)**	**Focal (n=38)**	**Diffuse (n=106)**
No lesion	161	153 (95%)	8 (5%)	0 (0%)
CIN 1	85	29 (34%)	29 (34%)	27 (32%)
CIN 2/3	80	0 (0%)	1 (1%)	79 (99 %)

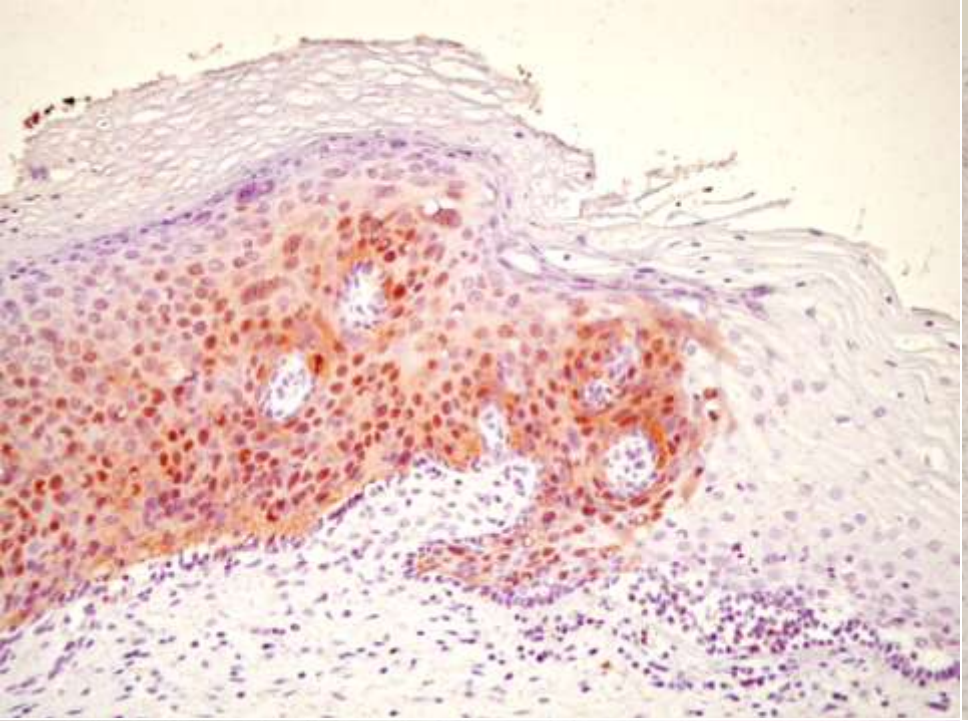
Ordi J, et al. *Int J Gynecol Pathol* 2009; 28:90-97

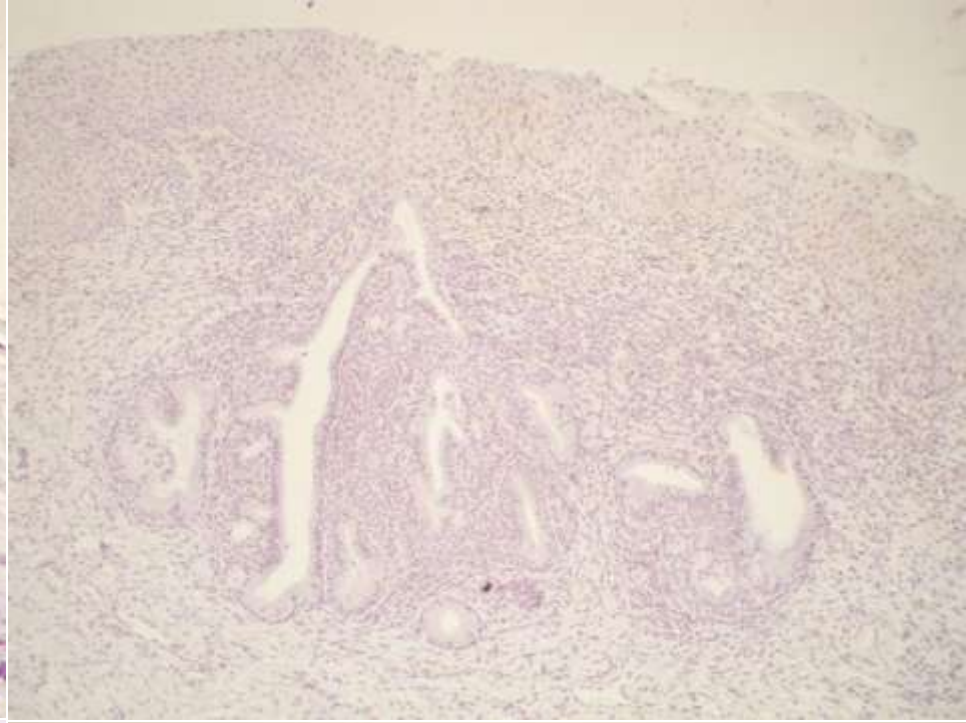


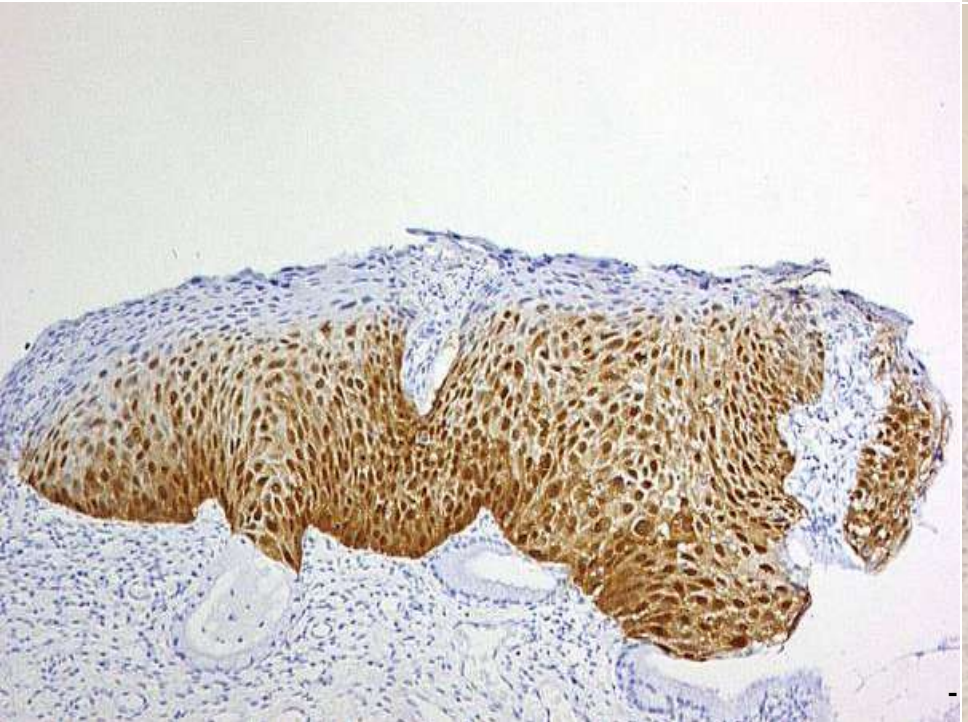


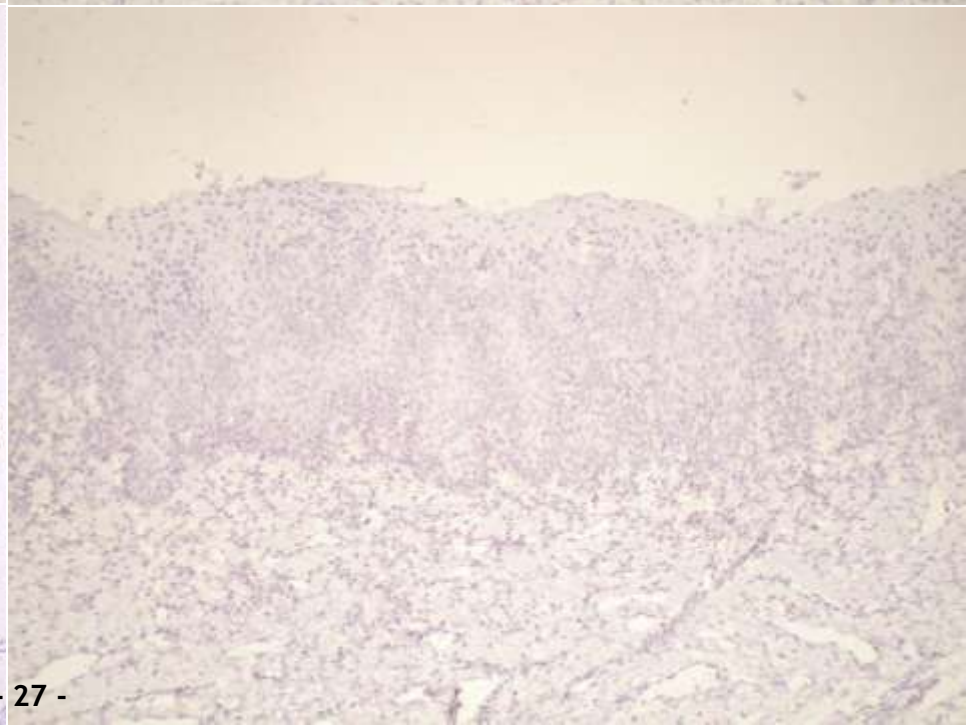


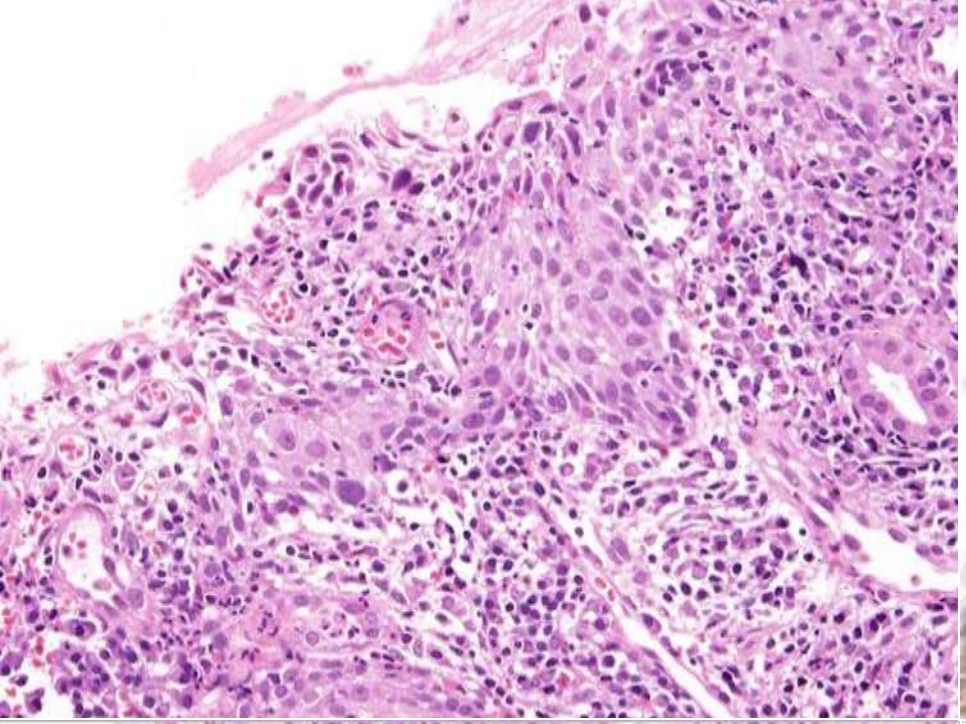
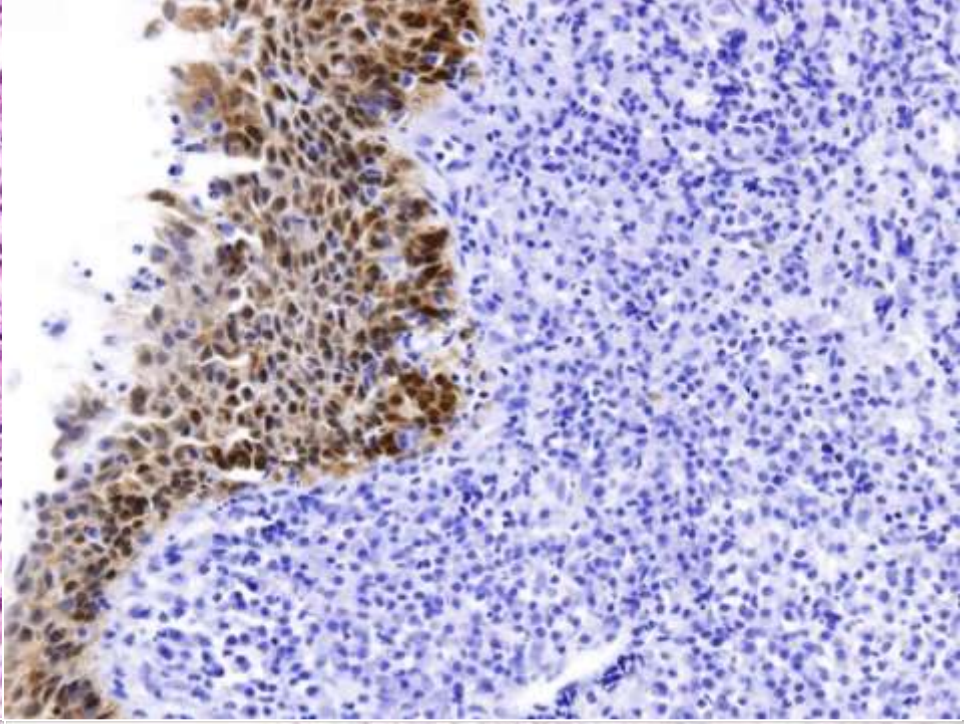
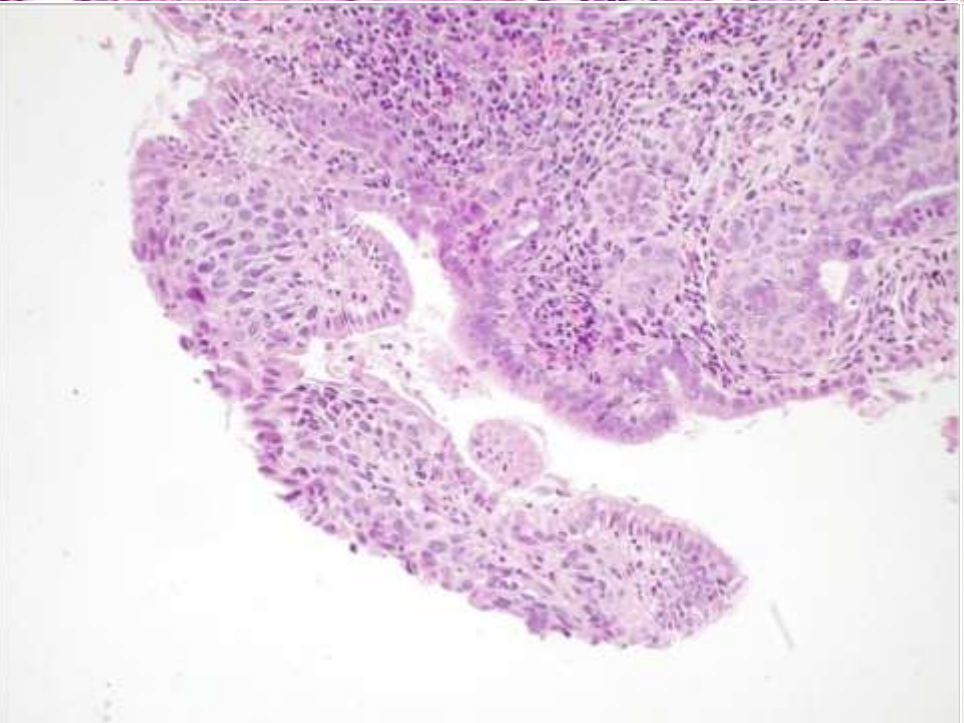
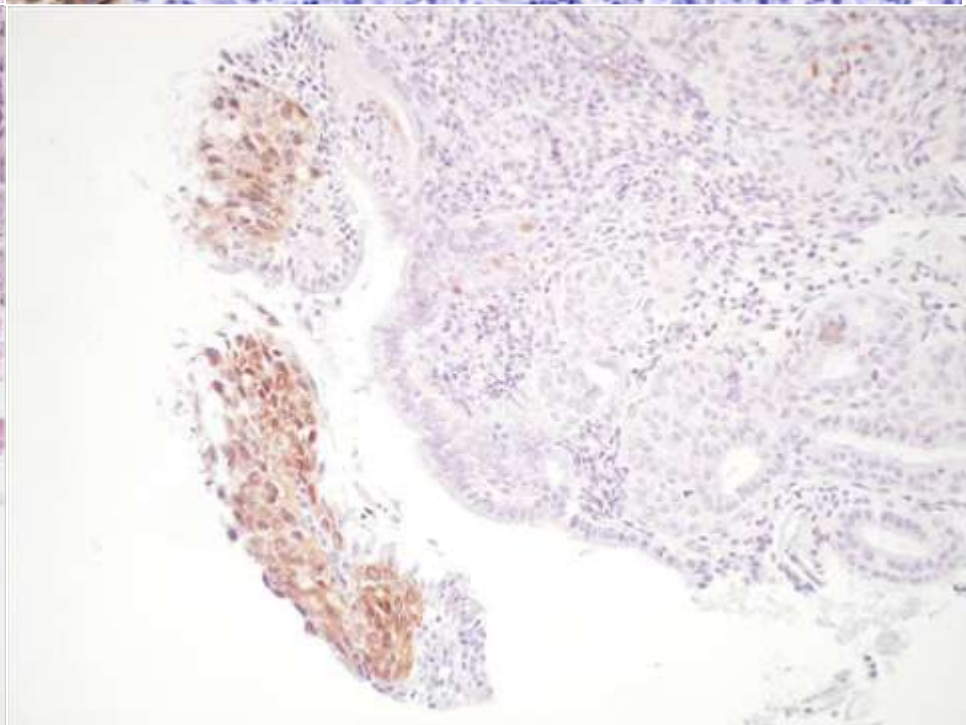


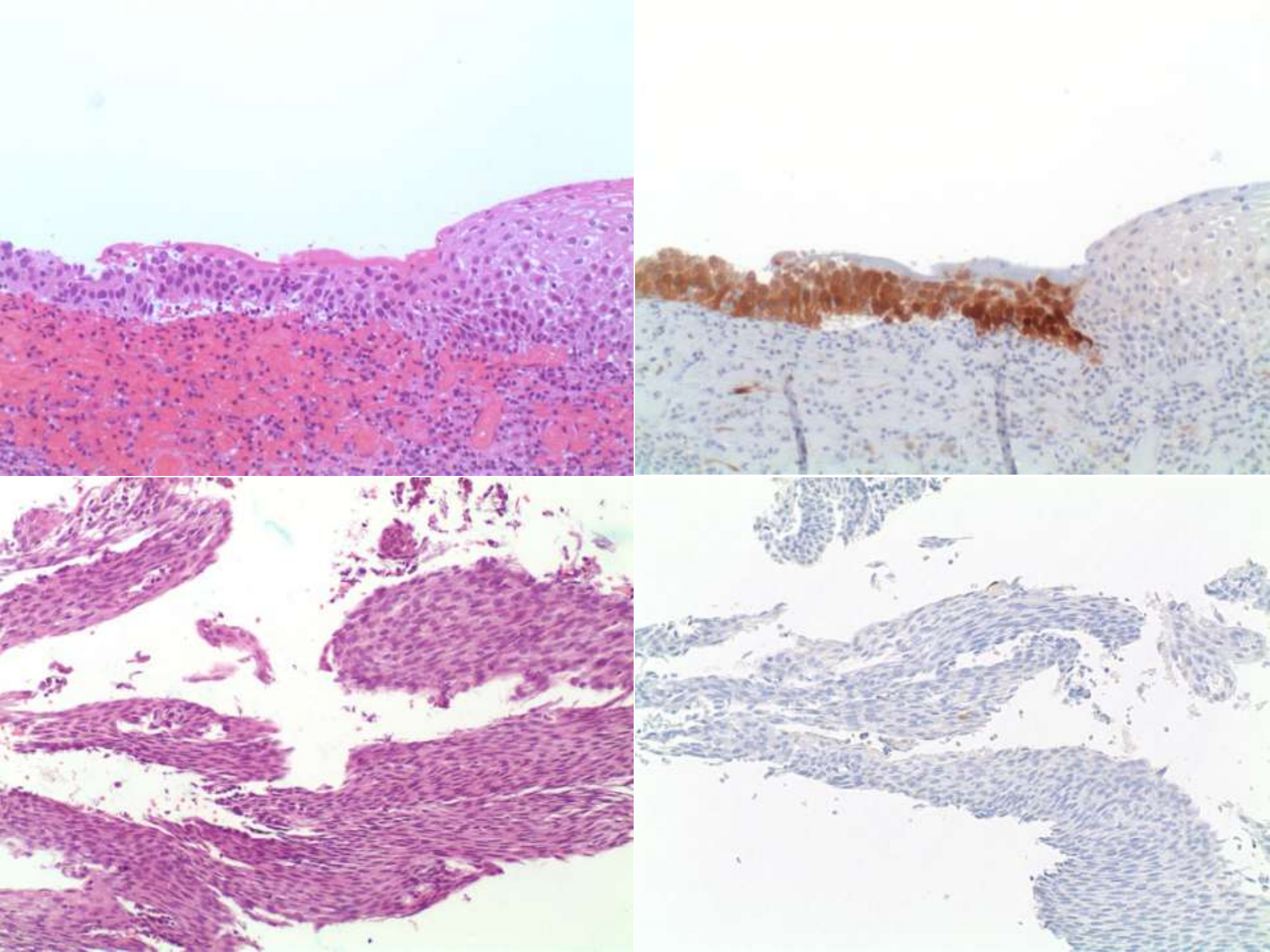


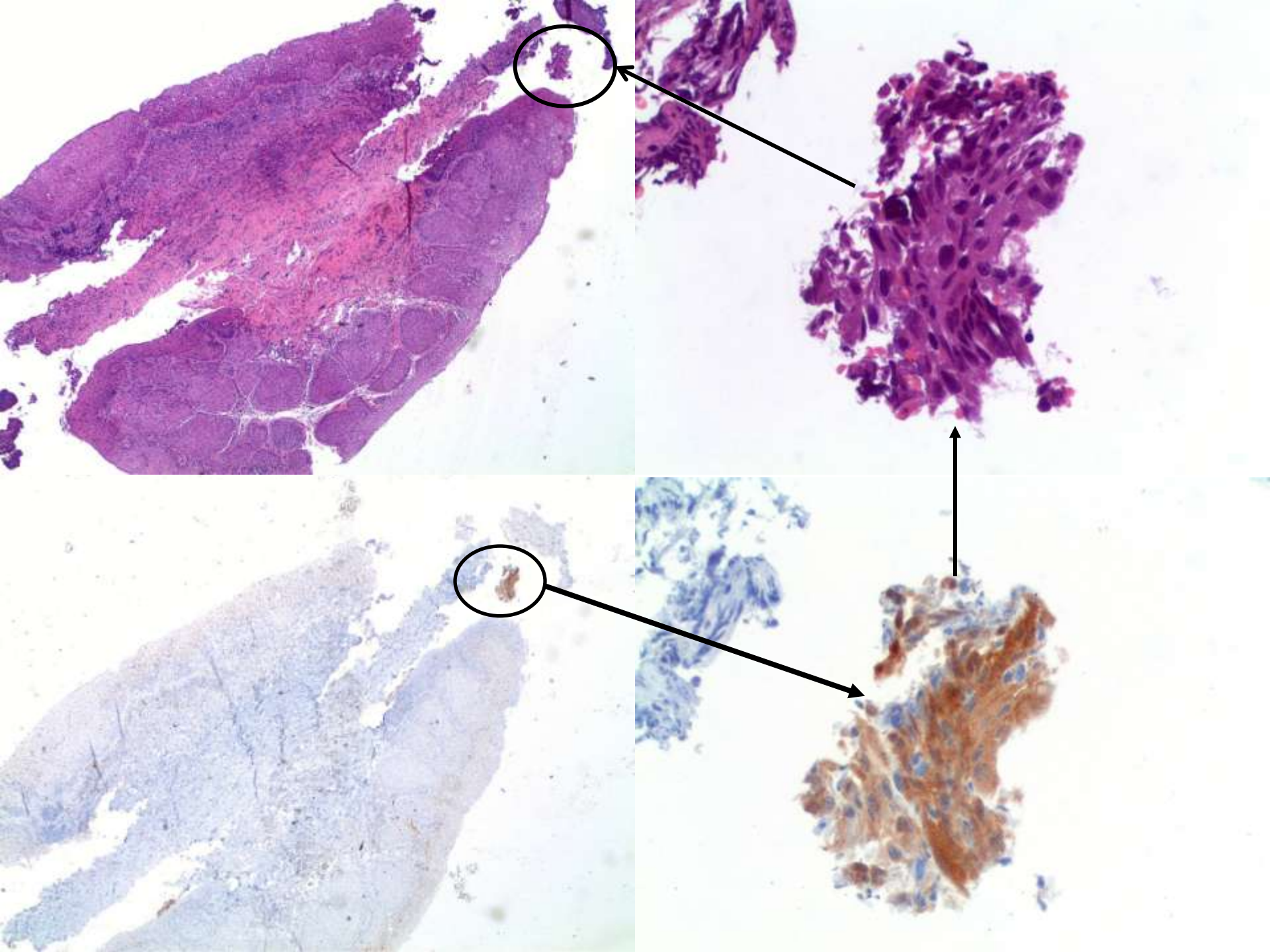


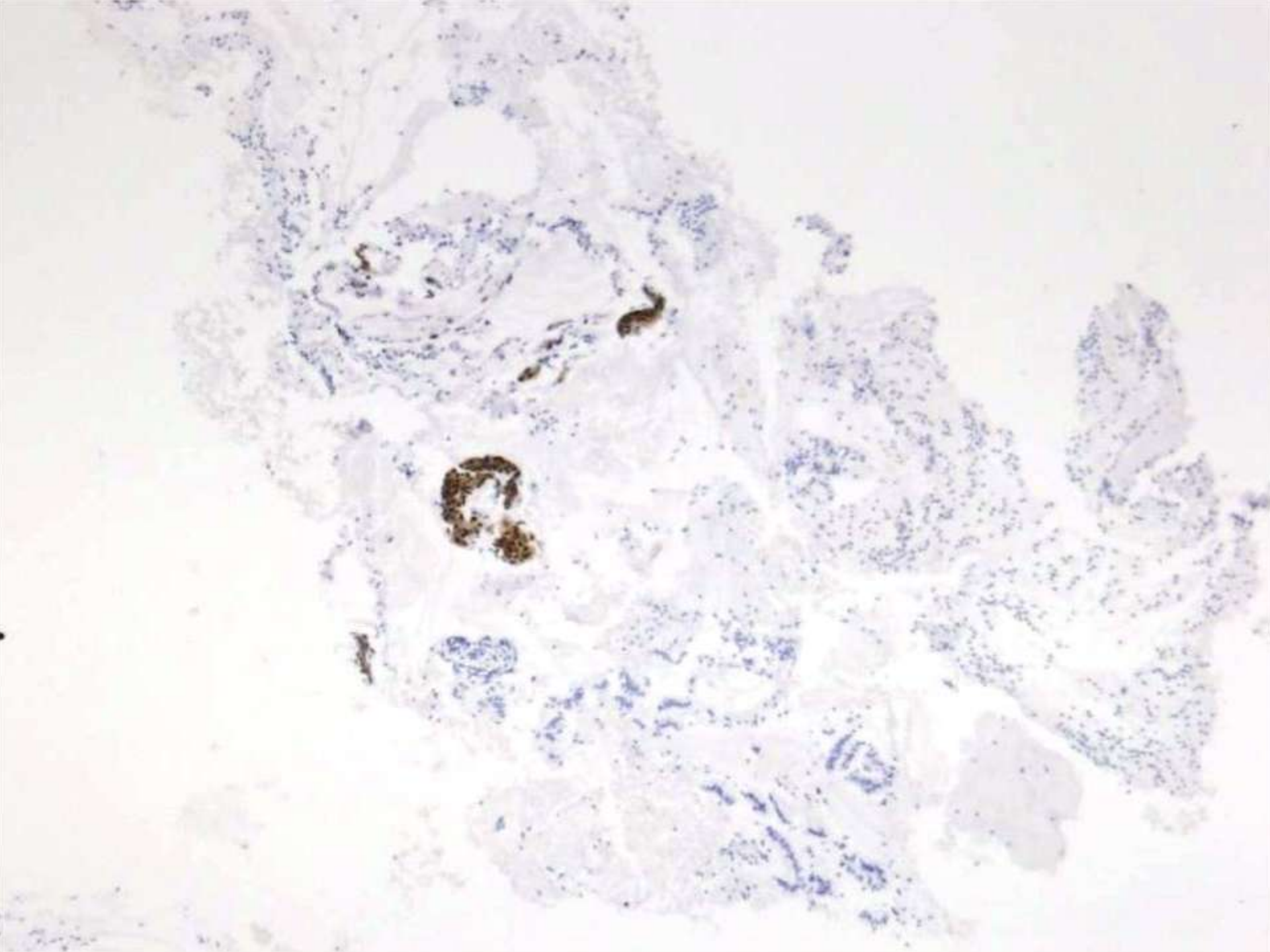




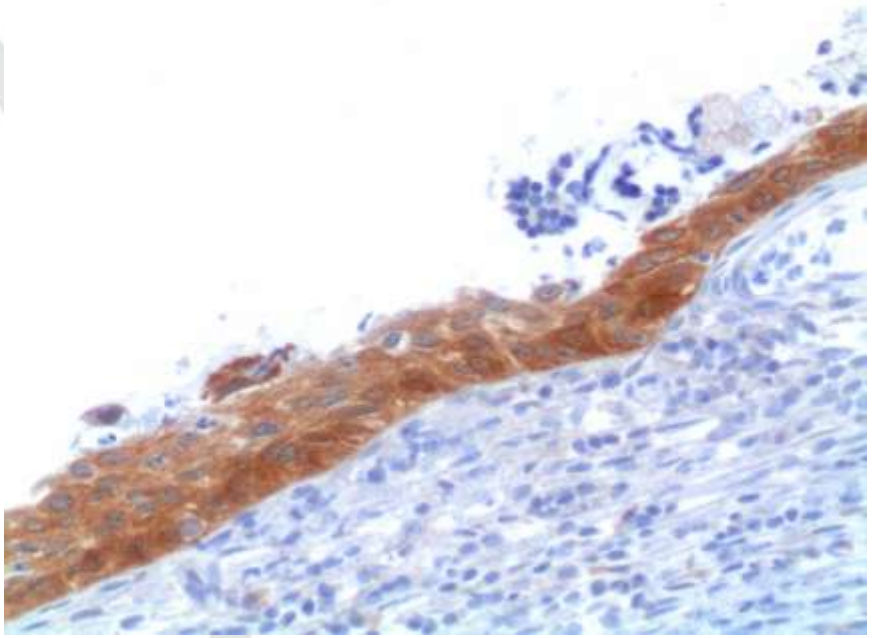






Conclusion

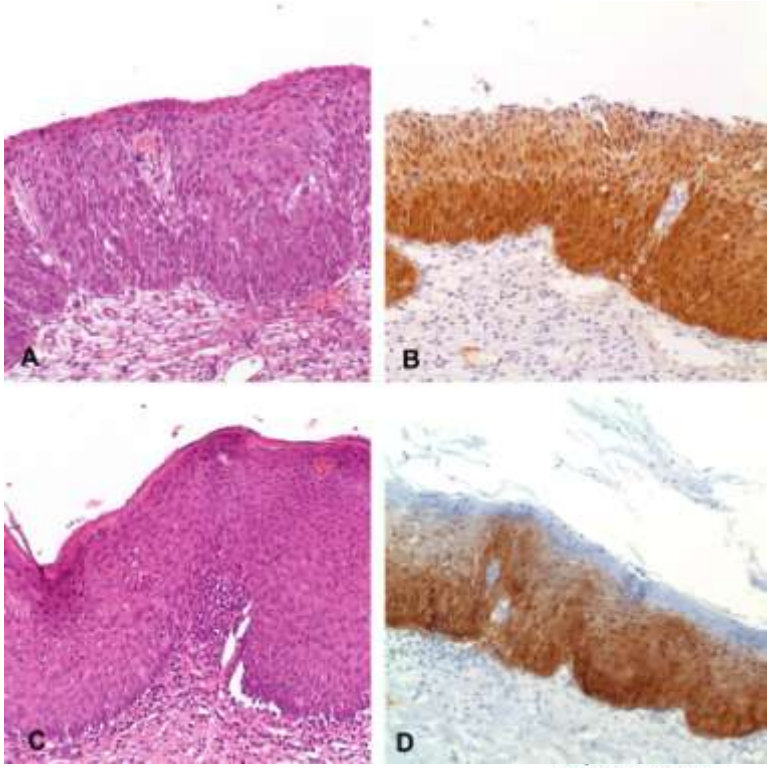
- $p16^{INK4a}$ immunostaining helps to identify a significant number of occult CIN lesions in HPV-positive women



CIN2-3, HC2 negative

- 646 women with a biopsy proven CIN2-3
- 20 (3.1%) with a negative HC2 test for HPV
- All of them positive for

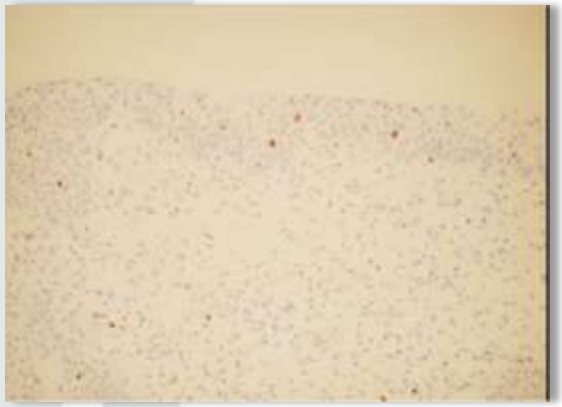
  p16

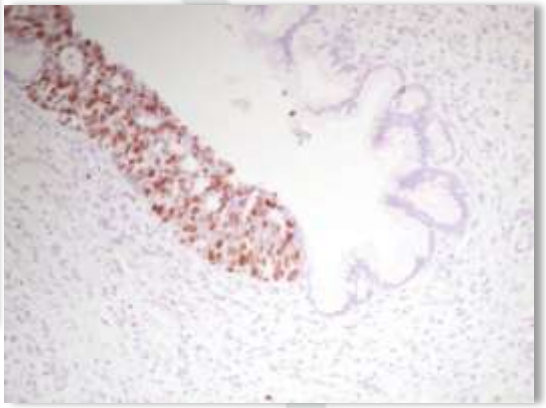


Del Pino M, et al. *Gynecol Oncol;* 2011, in press

Ki67 / p16 in cervical lesions

Diagnosis	p16				Ki-67			
	0	1	2	3	0	1	2	3
CIN1	10/12 (83.3%)	0	1/12 (8.3%)	1/12 (8.3%)	0	7/12 (58.3%)	5/12 (41.7%)	0
CIN2	0	3/6 (50.0%)	1/6 (16.7%)	2/6 (33.3%)	0	1/6 (16.7%)	5/6 (83.3%)	0
CIN3	0	0	0	13/13 (100%)	0	1/12 (8.3%)	5/12 (41.7%)	6/12 (50.0%)
p-value*	<0.001				0.003			





Nam EJ ,et al. *J Gynecol Oncol*. 2008; 19:162

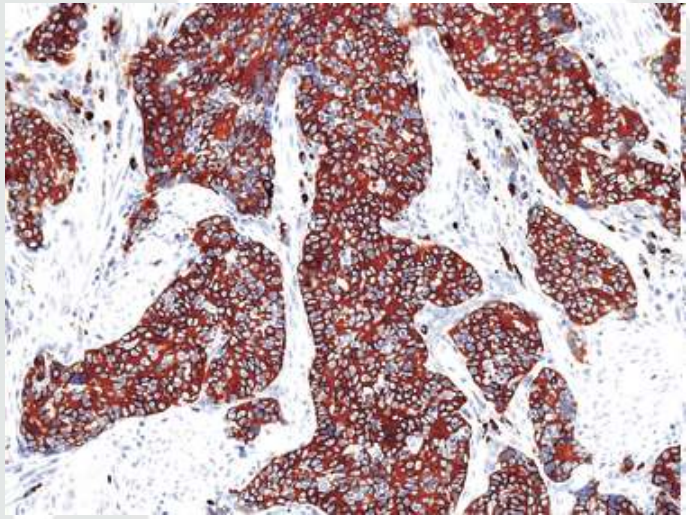
Ki67 / p16 in CIN3+

Biomarker	Sensitivity	Specificity
p16	99.2%	74.8%
Ki67	62.1%	94.9%
p16 + Ki67	99.2%	73.9%
HPV L1 antigen	16.5%	84.2%

Galgano MT ,et al. *Am J Surg Pathol* 2010; 34:1077

p16 and nm23

Biomarker	Sensitivity	Specificity
p16	96.3%	66.0%
nm23	81.8%	36.4%



Benevolo M ,et al.
Histopathology 2010; 57:580

p16, MCM2, TOP2A

Biomarker	H-SIL	L-SIL
p16	100%	76%
MCM2	79%	76%
TOP2A	86%	76%

Shih J, et al. *Hum Pathol* 2007; 38: 1335

ProEx C

Study	Sensitivity	Specificity
Shih (*Hum Pathol* 2007)	79%	28%
Pinto (*Mod Pathol* 2008)	87%	71%
Badr (*Am J Surg Pathol* 2008)	92%	80%
Sanati (*Int J Gynecol Pathol* 2010)	89%	100%

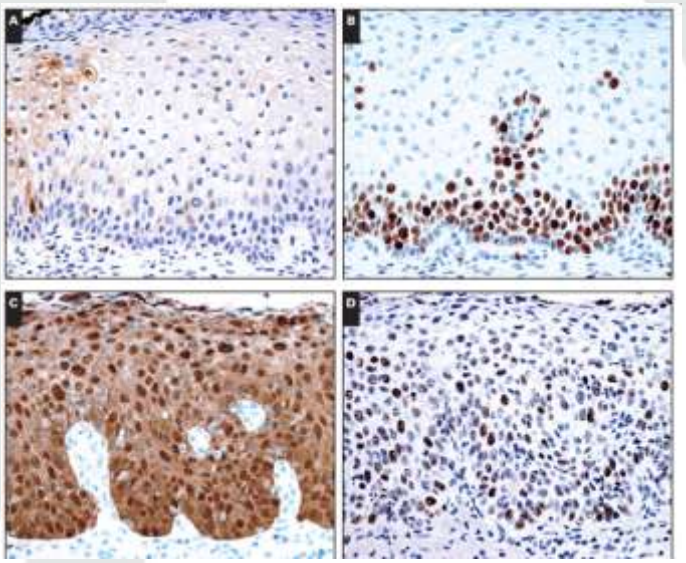
p16 vs. ProEx C

	CIN II/III		
	ProEx C+	p16 Band	Ki67+
Sensitivity (%)	92	97	89
Specificity (%)	80	90	88
PPV (%)	74	86	82.5
NPV (%)	94	98	93

Badr RE, et al. *Am J Surg Pathol* 2008; 32:899

p16 vs. ProEx C

Biomarker	H-SIL	L-SIL
p16	100%	76%
ProEx C	79%	94%
p16+ProEx C	100%	100%



Shih J, et al. *Hum Pathol* 2007; 38: 1335

p16 vs. ProEx C

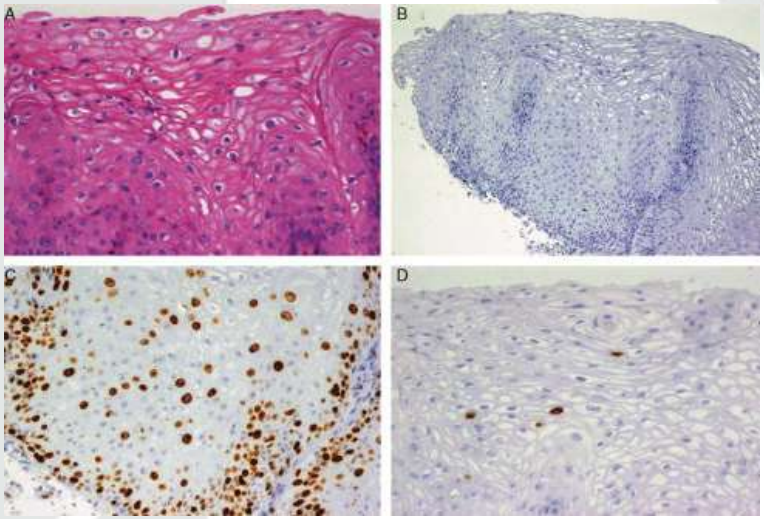
Biomarker	Sensitivity	Specificity
p16 (CIN2+)	79%	85%
ProEx C (CIN2+)	67%	85%
p16 (CIN3+)	90%	71%
ProEx C (CIN3+)	84%	79%
p16 + ProEx C (CIN2+)	57%	100%
p16 + ProEx C (CIN3+)	75%	93%

Guo M, et al. *Am J Clin Pathol* 2011; 135:212

p16 vs. ISH HPV

Diagnosis	Ventana HPVII ISH (%)	Ventana HPVIII ISH (%)	DakoCytomation ISH (%)	$p16^{INK4A}$ (%) Diffuse Strong	$p16^{INK4A}$ (%) Focal Strong and Diffuse Strong
HSIL	7/16 (43.8)	11/15 (73.3)	6/15 (40.0)	16/16 (100%)	16/16 (100%)
LSIL	8/12 (66.7)	7/11 (63.6)	9/12 (75.0)	7/12 (58.3)	11/12 (91.7)
Overall SIL	15/28 (53.6)	18/26 (69.2)	15/27 (55.6)	23/28 (82.1)	27/28 (96.4)

Diagnosis	Ventana HPVIII ISH (%)	DakoCytomation ISH (%)	$p16^{INK4A}$ (%) (Diffuse Strong)	$p16^{INK4A}$ (%) (Focal Strong and Diffuse Strong)	$p16^{INK4A}$ (%) (Diffuse Strong and Focal Strong With ISH+)
Sensitivity	69.2 (18/26)	55.6 (15/27)	82.1 (23/28)	96.4 (27/28)	92.9 (26/28)
Specificity	100 (30/30)	100 (30/30)	100 (30/30)	93.3 (28/30)	100 (30/30)



Kong CS, et al. *Am J Surg Pathol*; 2007; 31: 33

Conclusiones

- **p16** es el mejor marcador de lesiones de alto grado
- Reduce de la variación inter e intra- observador en el diagnóstico de CIN2-3
- Mejora de la sensibilidad del patólogo en la identificación de lesiones de CIN2-3
- La combinación con otros marcadores añade probablemente solo una información marginal

¿Podemos identificar lesiones de bajo grado con mayor riesgo de progresión?

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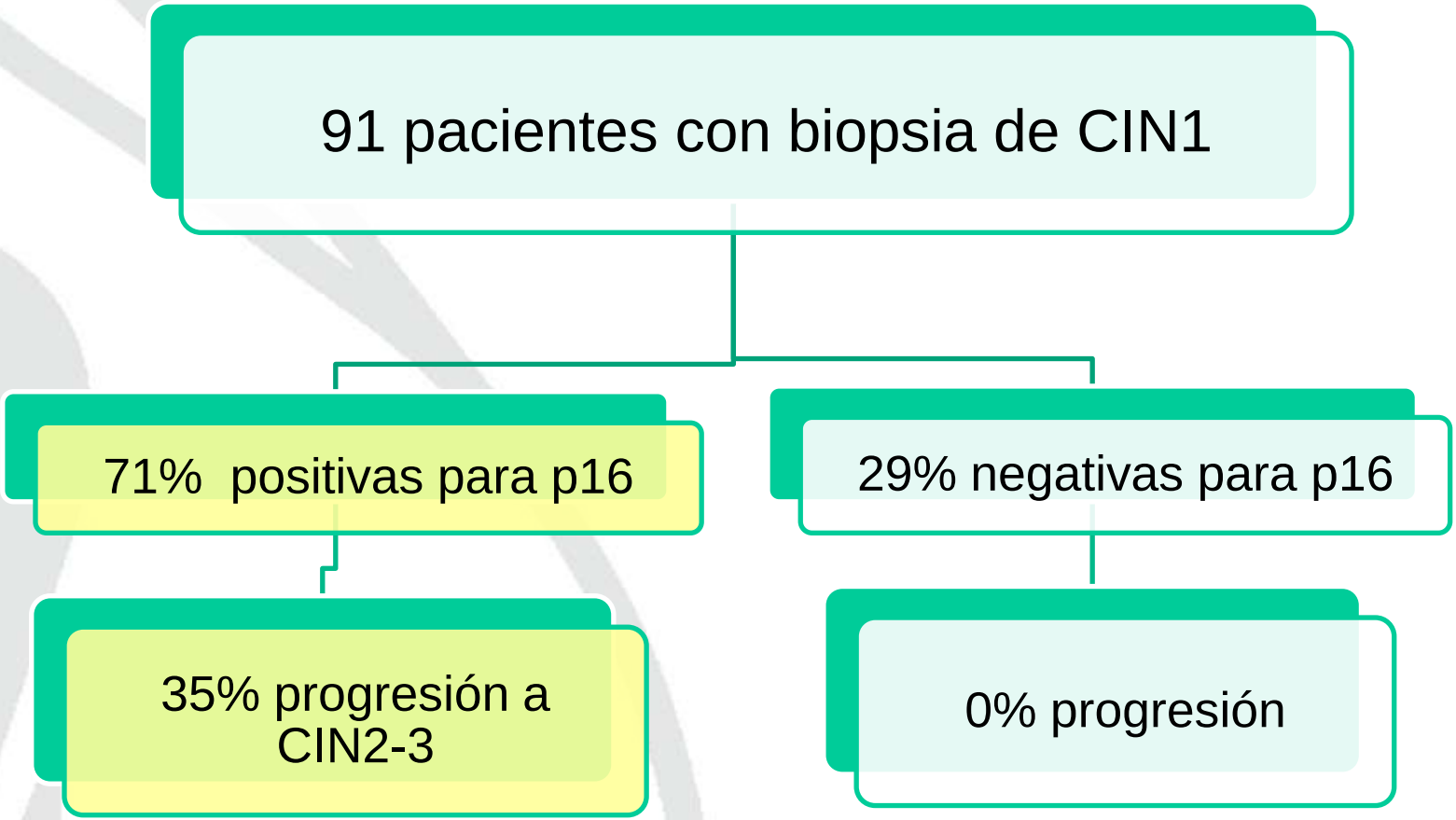
P16^{INK4a}: marcador de progresión



Hariri J et al. *Int J Gynecol Pathol*; 2007; 2223: 488

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P16^{INK4a}: marcador de progresión

- Mujeres con:
  - Biopsia de CIN1
  - Detección de VPH (HC2)
  - **Seguimiento durante al menos 12 meses con citología, colposcopia y VPH**
- 138 mujeres
- **Tinción para p16^{INK4a} en la biopsia**

Del Pino et al. *Am J Obstet Gynecol*; 2009; 201: 488

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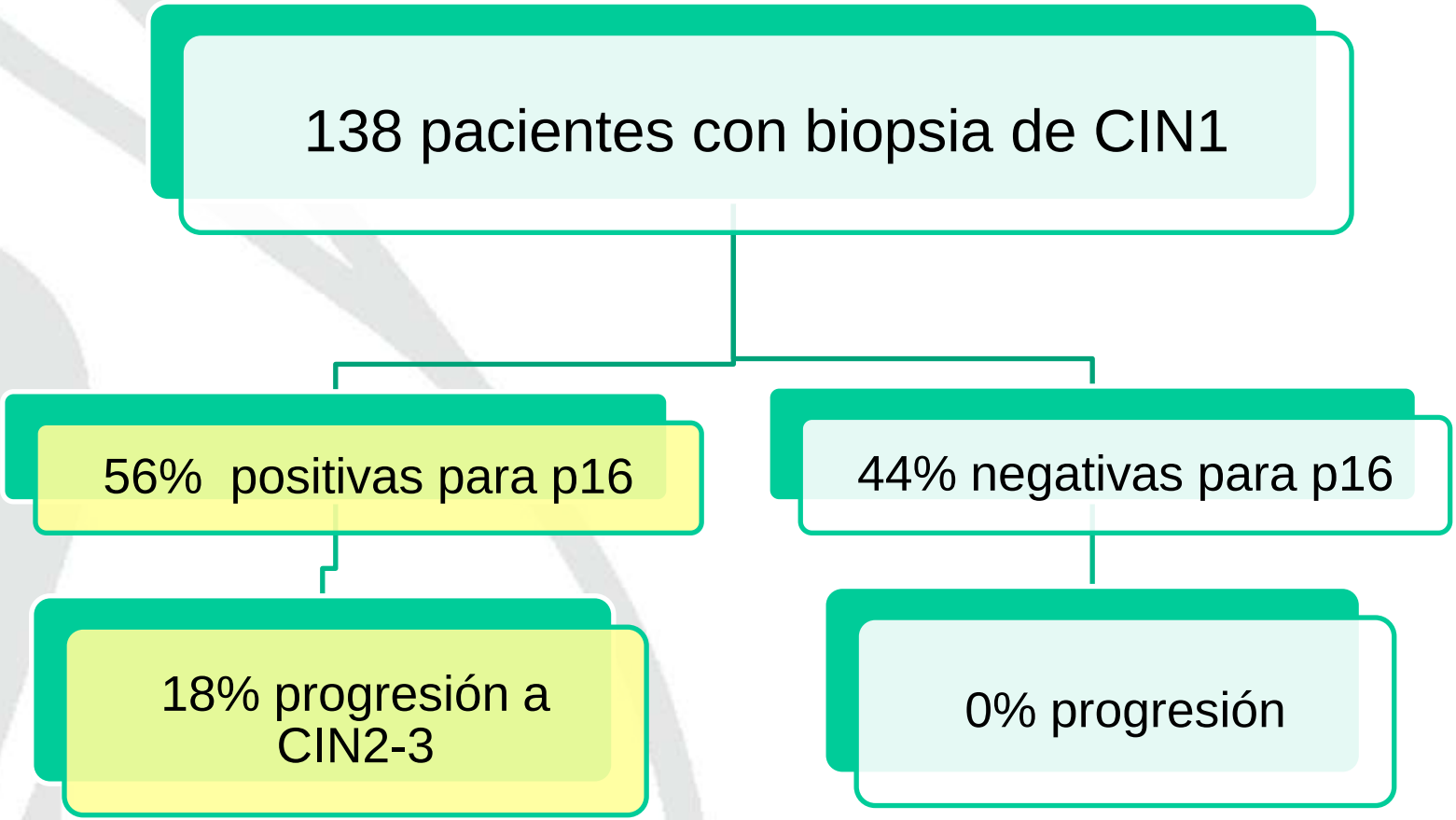
P16^{INK4a}: marcador de progresión



Del Pino et al. *Am J Obstet Gynecol*; 2009; 201: 488

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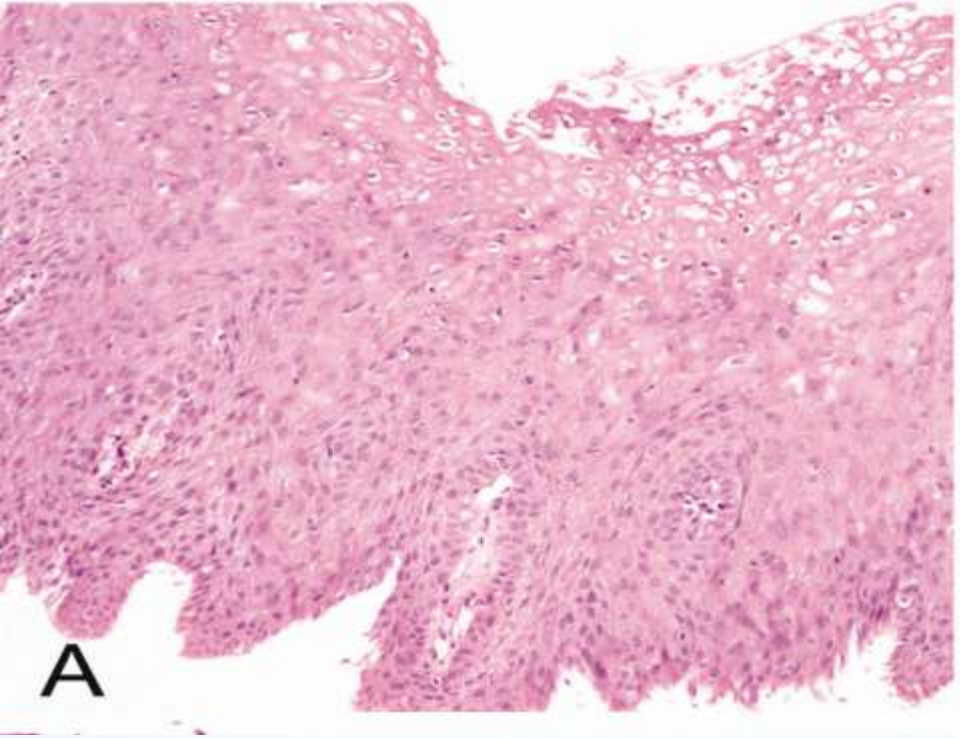
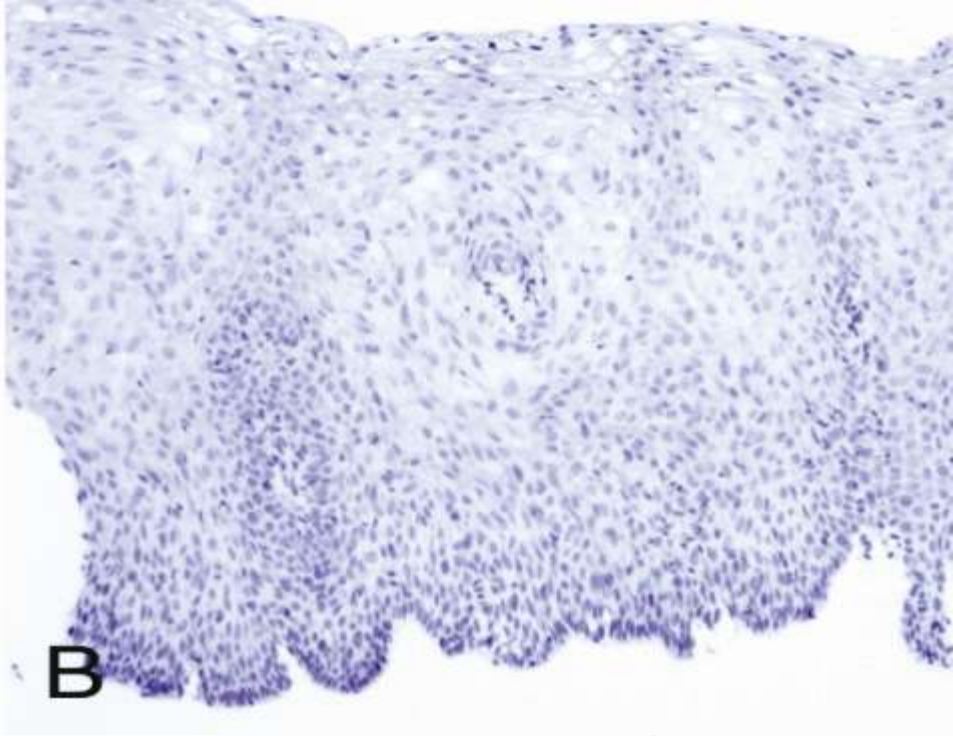

A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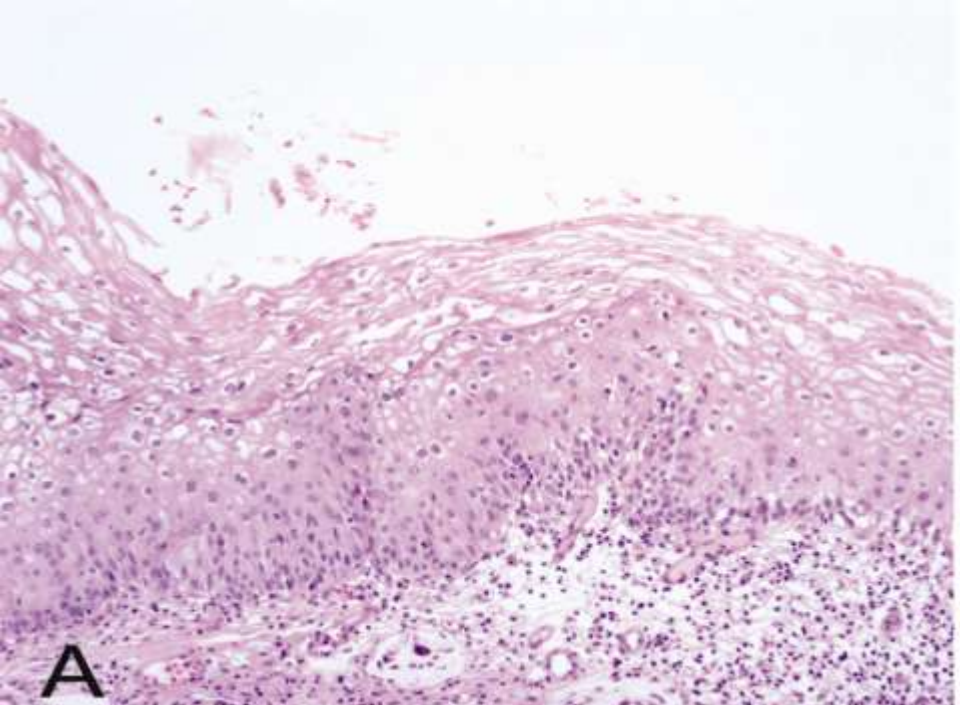
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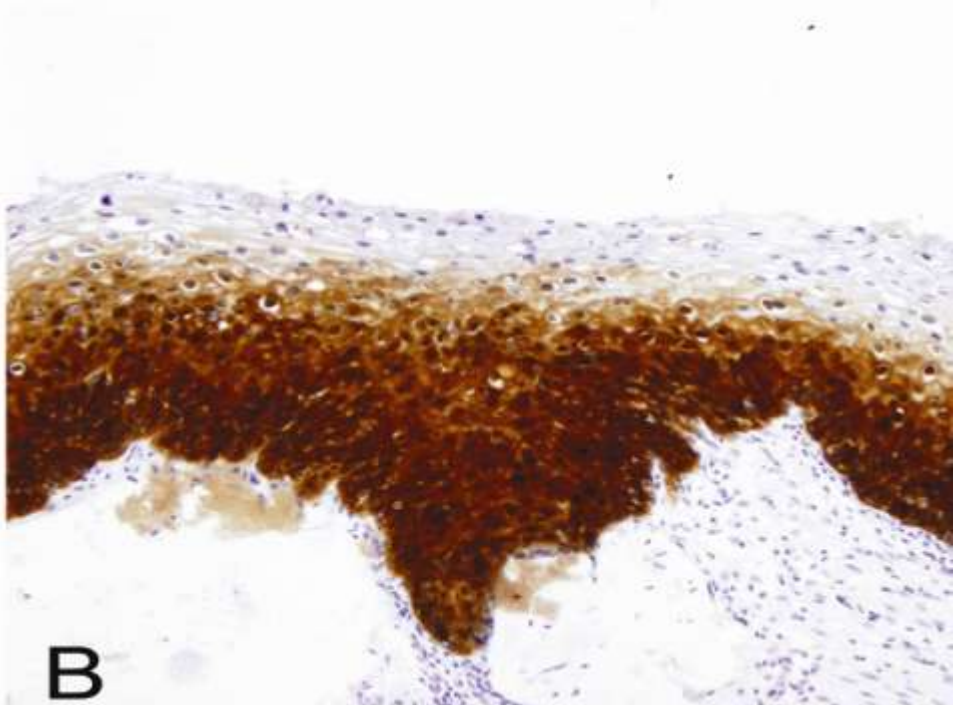

B

Conclusiones

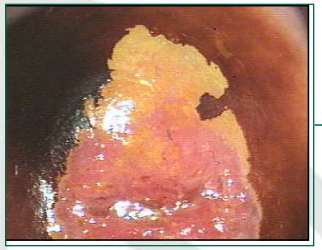
- Las paciientes con lesiones de CIN1 positivas para $p16^{INK4a}$ tienen un riesgo de progresión mas elevado
- Las lesiones de CIN1 negativas para $p16^{INK4a}$ raramente progresan y pueden beneficiarse de un seguimiento clínico menos intensivo

Conclusiones finales

- **Reducción de la variación inter e intra-observador en el diagnóstico de CIN2-3**
- **Mejora de la sensibilidad del patólogo en la identificación de lesiones de CIN2-3**
- Identificación de lesiones de CIN1 con mayor riesgo de progresión

Recomendaciones

- p16^{INK4a} como único marcador
- ¿Cuando?
  - **Escenario maximalista:** en toda biopsia cervical con sospecha clínica de displasia
  - **Escenario realista:** Siempre que existan dudas diagnósticas o discrepancias con el resultado citológico o virológico



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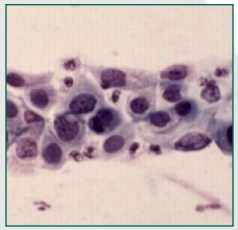

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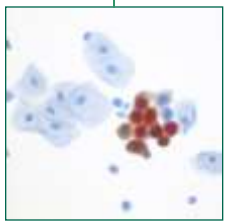

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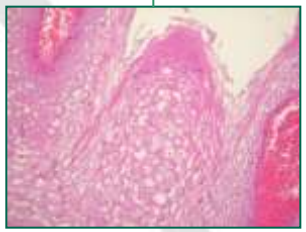

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Diagnóstico histológico
Resultado citológico
Resultado virológico

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